

Antimalarial, Antitrypanosomal, Antimicrobial Activities and Volatile Oil Profile of *Xylopiya aethiopica* (Dunal) Rich (Annonaceae)

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Abstract: The resistance of *Plasmodium falciparum* (*P. falciparum*) to well-recognized antimalarial drugs is now common in nearly every area of its prevalence; consequently, there is a need to explore therapeutic plants that have bioactive compounds which can be utilized in the manufacture of antimalarial drugs. The *in vitro* potency of the volatile oil (VO) of *Xylopiya aethiopica* (*X. aethiopica*) was evaluated against *P. falciparum* 3D7 strain, a human malaria parasite, a good activity against the parasite *P. falciparum* was recorded with an IC₅₀ of 14 µg/mL. The *in vitro* cytotoxicity of the fruit oil of *X. aethiopica* was determined at a concentration of 50 µg/mL against HeLa cells and was found to show no cytotoxicity effect at this concentration; this could be an indication of the safety of the fruit oil as a targeted drug for mammalian organisms. The antibacterial property of the VO from the fruit of *X. aethiopica*, was evaluated *in vitro*; the fruit oil exhibited good antimicrobial activity against the tested microorganisms, with *Klebsiella pneumoniae* having the highest inhibition zone of 19.0 ± 1.4 mm. The result recorded for MBC/MFC confirms that VO of this plant can be used in therapeutic preparations, particularly in the infections brought about by some of these microorganisms as practiced by traditional healers in folkloric medicines. This appears to justify the numerous folkloric therapeutic uses of the aerial parts of this plant. The hydro distilled volatile oil from *X. aethiopica* was characterized by Gas Chromatography-Mass Spectrometry (GC-MS), an abundance of monoterpenes hydrocarbon (58.0%), followed by oxygenated monoterpenes (30.13%), sesquiterpene hydrocarbon (8.96%), and fatty acid (0.74%) were well represented. The significant quantitative constituents of the fruit oil are 1, 8-cineole (16.73%), β-pinene (11.59%), sabinene (10.76%), 1R-α-pinene (8.30%), α-phellandrene (8.34%).

Keywords: *Xylopiya aethiopica*; antimalarial; antimicrobial screening; hydro distillation; volatile oil.

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1. Introduction

Great emphasis has been placed on herbal drugs used for human health care by the World Health Organization (WHO); this organization has printed different policies guiding principles and criteria for these botanical drugs [1]. Investigation into bioactive compounds obtained from plants as a source of alternative medicine to synthetic drugs have received much awareness and attention from natural product chemist all over the globe; this is because therapeutic plants have been discovered to possess several medicinal properties useful in the management of many ailments with little side effect when put side by side with synthetic drugs [2]. The main cause of malaria infection is the *Plasmodium* parasite; they contaminate the red blood cells (RBC) and bring about symptoms like pain, chills, fever, and sometimes swelling.

Many modern drugs like Artemisinin and Quinine are derived from a plant used to treat malaria [3]. The problem of resistance to some known drugs, particularly chloroquine, is one of the greatest problems about malaria control in this present day time [4, 5]. Chloroquine which was very cheap and effective against malaria parasites in the past, is now considered ineffectual due to multiple resistant poses by *P. falciparum* to this drug [6]. As a result of this resistance menace, malaria eradication is becoming increasingly difficult since the disease usually resurfaces in areas where it had been originally stamped out and emerges in formally unaffected vicinities. The resistance of *P. falciparum* to well-recognized antimalarial drugs is now common in nearly every area of its prevalence [7]. Therefore, there is a call for serious efforts to develop a new antiplasmodial drug from plants; all researchers of natural products are thereby encouraged to exert their precious time and energy into medicinal plants with antimalarial potencies by screening a large number of therapeutic plants from different tribes and rural areas where these medicinal plants have been reported to have great efficacy against *P. falciparum* parasite. If the antiplasmodial effect of these plants can be established after the necessary screening, the bioactive compounds responsible for the antiplasmodial activity can be isolated via a range of extraction methods.

Xylopiya aethiopica is a sweet-smelling therapeutic plant frequently found in the extreme forest-Savanna zone of Africa or the lowland rainforest region of Africa; this plant can reach about twenty meters high [8] and is classified among the Annonaceae family [9]. This evergreen plant is commonly seen in African countries like Gambia, Kenya, Togo, Sierra Leone, Liberia, Burkina Faso, and Nigeria [10, 11]. The fruit of *X. aethiopica* is greenish when freshly harvested but brownish dark when dried; the peppery taste and the strong “peppery note from the fresh fruit of *X. aethiopica* are largely due to “linalool” one of the volatile constituents of the oil, this constituent is responsible for the characteristic aroma of the ground, dried and smoked fruit of the plant [9]. The pods of this plant contain about 5-8 seeds; the fruit looks like cowpea pods with constriction along their length; the pod never split open to release its seeds. It is a plant of great repute in Africa, the wood obtained from this plant has been documented to be resistant to termite attack. As a result, the bark of the plant is used to make doors and partition, and also used in the construction of huts in rural African settings [12]. Barely every morphological part of *X. aethiopica*, like the leaves, stem, root, stem bark, and fruit (both dried and fresh), are used in traditional medicine to treat various diseases, including rheumatism, headache, malaria, asthma, epilepsy, infertility, dysentery, skin infections, candidiasis, ease of dyspepsia, cough, fever, and moderate anti-SARS-CoV-1 and SAR-CoV-2 infection [12-15]. Its anticancer, antimalarial, antidiabetic, antioxidant, enzyme inhibition, and antimicrobial potencies have been documented [13]. The plant is commonly referred to as African Pepper” (English name), “Ethiopian Pepper” or “Guinea Pepper, it is also referred to as ‘Eru’ in the South Western part of Nigeria [12].

Infusion obtained from the bark of the plant is employed in the treatment of bronchitis and dysenteric conditions or as a mouth wash to treat toothaches, used to reduce rheumatism pain [4], used as an insecticide to control insects, used in meat and food preservation, [10, 16], used in the management of neuralgia, headache, colic pain [17], to treat male infertility [18]. It was documented from previous research findings that extract derived from the fruit, when taken orally, can boost steroid hormone and sperm count [19]. Combination of extract from this plant with an extract from New-boudin leaves (Bignoniaceae) plant was used some time ago in hospitals owned by Ghana Government to enhance menstrual flow and induce placental discharge during post-partum due to its abortifacient properties [12, 17] while powered derived

from the root of *X. aethiopica* was used as dressing for sores and engaged in the local treatment of cancer [20]. A decoction from the root and leaves of this plant is commonly referred to as 'Agbo' in South-Western Nigeria, while the powder from the root, when combined with salt, is used to treat constipation [21]. The fruit obtained from *X. aethiopica* is an important ingredient in the Eastern part of Nigeria used to prepare local soup believed to aid the easy flow of breast milk from breastfeeding mothers. Different extracts from the aerial parts of *X. aethiopica* have been used as an adjunct therapy to treat sickle cell disease [22]. Thus, *X. aethiopica* is an essential item in Africa used majorly for spice, medicine, and flavoring.

As a result of the efficient antimicrobial activities displayed by *X. aethiopica* in traditional settings, researchers embarked on the *in vitro* antimicrobial test to ascertain these claims. Konning *et al.* [23] investigated the antimicrobial efficacy of the methanol extract of this plant and found out that the extract was active against both Gram-positive and Gram-negative bacteria with excellent antifungal activity against *Candida albicans* and *Aspergillus niger* while Alfred *et al.* [24] worked on the antimicrobial activity of the dichloromethane/methanol extract from the seed of the plant, it was documented by this researcher that the extract showed positive activity against *S. aureus* ATCC 25923, *C. albicans* ATCC 10239, *E. coli* ATCC 25922, *C. violaceum* CV 12472 and *P. aeruginosa* PA 01 with same MIC values of 0.625 mg/mL for *S. aureus* ATCC 25923, *P. aeruginosa* PA 01 and *C. albicans* ATCC 10239, and same MIC values of 2.5 mg/mL for *E. coli* ATCC 25922 and *C. violaceum* CV 12472. Several reports of the antimicrobial activity of the volatile oil from the plant of study have been reported in the literature; the volatile oil, as well as the crude extract (both alcoholic and aqueous) of the plant, have been shown to have a wide range of activity against Gram-positive, Gram-negative bacteria and *Candida albicans* [18, 25-27].

The commonest bioactive compound present in the different parts of *X. aethiopica* is the terpene constituents found in the volatile oil of the plant [6]. Several studies have been carried out on the volatile oils from this plant; they have displayed the presence of a broad diversity of chemical compounds. In one such study conducted in Togo thirty-five constituents were found in the volatile oil with major constituents as β -pinene (23.6%), α -pinene (11.0%), sabinene (9.80%), germacrene D (8.30%), and 1,8-cineole (8.20%) [28], while the oil from Mali was rich in β -pinene (19.1%), γ -terpinene (14.70%), trans-pinocarveol (8.60%) and p -cymene (7.30%) [29].

Therefore this present work seeks to evaluate the volatile oil from the crude extract of *X. aethiopica* fruit oil as an alternative herbal medicine to curb plasmodial parasite and some microbial diseases; since this plant is used in folk medicine for various therapeutic purposes, their volatile oil is likely the reasons for their different medicinal values.

2. Materials and Methods

2.1. Plant material and essential oil isolation.

The matured seeds of *X. aethiopica* were obtained from their natural habitat in the Ogugu area of Kogi State of Nigeria. Identification was made at the Department of Botany, Kogi State University, Anyigba, Nigeria. Voucher specimen was deposited at the University herbarium. The fruits were macerated using a fast rotating blender and subjected to hydro-distillation using Clevenger-type apparatus. GC-MS analysis was immediately carried out on the extracted volatile oil.

2.2. Gas chromatography/mass spectrometry analysis.

The fruit oil of *X. aethiopica* was analyzed using multidimensional gas chromatography coupled with a gas chromatography-mass spectrophotometer (Shimadzu Japan, 2010) equipped with non-polar and polar double capillary columns (25.0m×0.25µm i.d., 0.25µm df). The carrier gas used was helium of high-level purity at a constant flow rate of 0.99 mL/min. The oil sample of 1 µL volume was injected at a split ratio of 41.6 into the GC and GC/MS with an AOCZOi auto-injector. The starting temperature of the instrument was set at 60 °C (held for 1 min), heated at a rate of 10°C/min to 180°C, held isothermally for 3 minutes, and then at 12°C/min to 280°C (finally held for 2 min). Ion source and transfer line temperatures were set to 200 °C and 250 °C, respectively, while a solvent delay of 3 minutes was observed. Electron impact (EI) ionization mode was 70 EV, and the linear velocity of the column was 36.2 cm/sec. Based on their mass spectra, various components were identified compared with those available in the NIST Library Mass Spectra database and mass spectra from literature. [30].

2.3. Tested bacteria and fungus strains.

Escherichia coli (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*) *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhi* (*S. typhi*) (Gram-negative bacteria), *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*) (Gram-positive bacteria and *Candida albicans* (*C. albicans*) (Yeast like fungus). These organisms were collected on sterilized nutrient agar slants, and incubation was carried out at 37°C for a day.

2.4. Antimicrobial screening.

2.4.1. Zone of inhibition-agar well diffusion method.

The antimicrobial efficacy of the volatile oil of *X. aethiopica* was determined using seven pathogenic microorganisms (clinical bacterial isolates) obtained from the Microbiology Section (Environmental Technology Division) of the National Research Institute for Chemical Technology, Zaria. According to the National Committee for Clinical Laboratory Standard [31], Agar well diffusion method was used to check the antimicrobial activities of the volatile oil extract. Nutrient agar of about 30 mL in different Petri dishes was swabbed via a 24 h sub-cultured bacteria, which were prepared in dilution to match the turbidity intensity of the McFarland standard. Wells (6 mm diameter) were punched in the agar and filled with 10 µL of different concentrations (100, 80, 60, and 40 µg mL⁻¹) obtained from the stock solution prepared by dissolving 20 mg/mL of the volatile oil (VO) in Dimethyl Sulfoxide (DMSO). The plates were incubated at 37°C for 24 h. The antibacterial and antifungal activities were accessed by measuring the zone of inhibition in diameter (mm) around the well. DMSO was used as a negative control.

2.4.2. Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/ Fungicidal Concentration (MBC/MFC).

Determination of the MIC was carried out using the micro-tube dilution method according to the method described by Collin *et al.* [32]. A dilution series of the extract, ranging from 20-100 µg mL⁻¹, were prepared and then transferred to the broth in micro-tube. Before

inoculation of the test organisms, the bacterial and yeast strains were adjusted to 0.5 McFarland. Each micro-tube was labeled appropriately and incubated at 37°C for 18-24 h for bacteria and 25°C for 48 h for the yeast cultures. The MIC values of the extracts were described as the lowest concentration that exhibited no noticeable growth when put side by side with the control containing no extract as the antimicrobial agent. The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by plating samples from clear micro-tubes showing no growth onto Mueller Hinton Agar and potato dextrose agar (PDA), respectively. MBC and MFC were defined as the lowest concentration yielding negative subculture. Both Ciprofloxacin and Ketoconazole were used as the standard antibacterial and antifungal agents (positive control) [33].

2.5. Antimalarial activity and cell cytotoxicity.

Investigation into the toxicity of the volatile oil of *X. aethiopica* was carried out by using human cervix adenocarcinoma cells (HeLa cells) according to the method described by Keusch *et al.* [34]. The volatile oil (20 mg/mL) was prepared in DMSO, this was diluted with culture medium to 50 µg/mL, and 50 % inhibitory concentration from the dose-response assay was obtained from the single concentration assay of the volatile oil, which greatly decreases parasite viability to less than 20 %. In brief, the volatile oil was serially diluted while the concentration capable of inhibiting the 50 % cell viability was ascertained by non-linear regression of the dose-response curve. Cytotoxicity of the volatile oil was determined by incubating the oil in duplicate wells with about 1×10^4 HeLa cells per well; the duration for the incubation was 48 h carried out at 37 °C in 5% CO₂. The number of cells that outlived exposure to the volatile oil was found by employing resazurin-based reagent through resorufin fluorescence reading inside the multi-well plate reader. The viability was obtained by comparing the resorufin fluorescence reading of the compound in treated wells with untreated control [35].

The *pLDH* assay, as described by Makler *et al.* [36], was used for the antimalarial determination; the stock solutions for the screening of the volatile oil extract against malaria parasites were carried out the same way as described in the cytotoxicity assay. Fifty (50 µg/mL) of the oil extract was added with parasite culture in a 96- well plate which was incubated for 48 h at 37 °C in a 5% CO₂ incubator. At the end of the 48 h, the *pLDH* assay was carried out by removing 20µL of the culture from each well and added to 125 µL of a combination of Malstat and nitrotriazolium blue chloride (NBT)/phenazineethosulphate (PES) solutions in a fresh 96-well plate. The purple color produced when *pLDH* is present is determined by absorbance at 620 nm, equivalent to the number of parasites in the well. The extract that can decrease parasite viability appreciably from the single concentration assay is further employed in a dose-response assay to determine IC₅₀ values.

2.6. Antitrypanosomal activity.

The efficacy of the volatile oil against the trypanosome parasite was accessed using *Trypanosoma brucei brucei* (*T.b brucei*) parasite; the stock solution was also prepared the same way as depicted in the cytotoxicity assay. Fifty µg/mL of the VO was added to the *in vitro* culture of *T.b brucei* in 96-well plates, and the resultant mixtures were incubated for 48 h; the number of parasites that are capable of withstanding the drug contact was calculated by adding

resazurin based reagent as explained earlier in the cytotoxicity assay. The reagent resazurin is usually reduced to resorufin by living cells. Pentamidine was used as a positive control [37].

2.7. Statistical analysis.

Statistical analysis data were analyzed using Microsoft Excel and reported as mean \pm standard deviation of triplicate determinations. In addition to this, the non-linear regression using Prism 5 for Windows, Version 5.02 (Graph Pad Software, Inc) program was used to resolve IC₅₀ from the dose-response curve [38].

3. Results and Discussion

3.1. Volatile oil components of *X. aethiopica*.

The result of the identified components of the fresh fruit of *X. aethiopica*, the percentage composition, the retention indexes, molecular formula, molecular weight, and similarity index are contained in Table 1. The chromatographic analysis revealed the occurrence of twenty-two constituents in the fruit oil of this plant, amounting to 97.33%. The oil is rich in monoterpenes hydrocarbons (58.0%), followed by oxygenated monoterpenes (30.13%), sesquiterpene hydrocarbons (8.46%), and fatty acids (0.74%). It is evident that the composition of the oil is different both qualitatively and quantitatively, as indicated in Table 1.

The significant quantitative constituents of the volatile fruit oil of this plant are 1, 8-cineole (16.73%), β -pinene (11.59%), sabinene (10.76%), 1R- α -pinene (8.30%), α -phellandrene (8.34%), 4-terpineol (6.96%) and γ -terpinene (6.08%). Other notable compounds found in the seed oil are 2-carene (4.65%), germacrene (4.27%), linalool (3.02%), γ -elemene (2.48%), α -terpineol (2.39%), and ocimene (2.10%). Compounds present in significant amount are terpinolene (1.99%), α -thujene (1.41%), aromandedrene (1.25%), etc. 1, 8-cineole is volatile oxygenated monoterpene ether with various levels of pharmacological effects, and it's employed as a marker for medicinal volatile oil classification [39], it is also known for its medicinal and flavoring properties [40]. Linalool, one of the volatile constituents of the seed oil of this plant, is accountable for the "intense pepperish taste" of the fresh fruit. A small portion of fatty acid (oleic acid) 0.74% was also discovered in the fruit oil, oleic acid (a mono-saturated omega-9-fatty acid) have several health benefits; it is used as a concentrate in cosmetics, decrease blood pressure, augment fat burning, defend the cell against free radical damage, and put off ulcerative colitis [30]. The result reported above for the fruit oil of *X. aethiopica* in our study is slightly different from what was observed in the analysis of the fruit oil from Togo where it was reported that β -Pinene (23.6%), α -Pinene (11.0%), sabinene (9.80%), germacrene D (8.30%) and 1,8-cineole (8.2%) are the major components [28], while in Mali the major components of the fruit volatile oil are β -pinene (19.1%), γ -terpinene (14.70%), trans-pinocarveol (8.60%) and p-cymene (7.30%). [29], but the present study reveals 1, 8-Cineole (16.73%), β -pinene (11.59%), sabinene (10.76%) α -Phellandrene (8.34%), 1R- α -Pinene (8.30%) as the major constituents (Table 1). It could be seen that each oil sample has its compositional pattern different from others and the reason is simply due to the different compositional patterns of volatile oils, which are affected by geographical and climatic conditions [41]. This point is also bolstered by Raphael *et al.* 2020 [10], who also documented different antioxidant values between *X. aethiopica* of Ghana and that of Nigeria species.

Table 1. Chemical profile of the Seed oil of *X. aethiopica*.

S/N	Constituents	RI	MF	MW	SI % to TC	Percentage (%)
1	Sabinene	897	C ₁₀ H ₁₆	136	95	10.76
2	α-Thujene	902	C ₁₀ H ₁₆	136	94	1.41
3	β-Pinene	943	C ₁₀ H ₁₆	136	96	11.59
4	1R-α-Pinene	948	C ₁₀ H ₁₆	136	97	8.30
5	α-Phellandrene	969	C ₁₀ H ₁₆	136	94	8.34
6	Ocimene	993	C ₁₀ H ₁₆	136	91	2.10
7	γ-Terpinene	998	C ₁₀ H ₁₆	136	94	6.08
8	2-Carene	1001	C ₁₀ H ₁₆	136	93	4.65
9	Terpinolene	1052	C ₁₀ H ₁₆	136	93	1.99
10	1,8-Cineole	1059	C ₁₀ H ₁₈ O	154	88	16.73
11	Linalool	1082	C ₁₀ H ₁₈ O	154	93	3.02
12	Trans-para-2-Menthen-1-ol	1109	C ₁₀ H ₁₈ O	154	84	0.55
13	4-Terpineol	1137	C ₁₀ H ₁₈ O	154	93	6.96
14	α-Terpineol	1143	C ₁₀ H ₁₈ O	154	94	2.39
15	Cis-β-Terpineol	1158	C ₁₀ H ₁₈ O	154	85	0.32
16	1,4-Dihydro-2-Methylbenzoic acid	1191	C ₈ H ₁₀ O ₂	138	83	2.78
17	(-)-Isopulegol	1196	C ₁₀ H ₁₈ O	154	83	0.16
18	Aromandrene	1386	C ₁₅ H ₂₄	204	89	1.25
19	γ-Elemene	1465	C ₁₅ H ₂₄	204	88	2.48
20	Germacrene D	1515	C ₁₅ H ₂₄	204	87	4.27
21	α-Caryophyllene	1579	C ₁₅ H ₂₄	204	95	0.46
22	Oleic acid	2175	C ₁₈ H ₃₄ O ₂	282	87	0.74

Note: SN= serial number, RI= retention index, MF= molecular formula, MW= molecular weight, SI= similarity index and TC= targeted compound.

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) values (µg/mL) for *Xylopiya aethiopica* fruit oil and the standard drug.

Microbial isolates tested	Fruit oil (sample)		Ciprofloxacin (Positive control)		Ketoconazole (Positive control)		(DMSO) (Negative Control)
	MIC	MBC/MFC	MIC	MBC	MIC	MFC	0.4 mL
<i>Escherichia coli</i>	ND	ND.	50	60	-	-	0.4 mL
<i>Klebsiella pneumoniae</i>	70	90	50	70	-	-	0.4 mL
<i>Pseudomonas aeruginosa</i>	80	80	50	60	-	-	0.4 mL
<i>Salmonella typhi</i>	80	90	40	50	-	-	0.4 mL
<i>Bacillus subtilis</i>	80	90	50	60	-	-	0.4 mL
<i>Staphylococcus aureus</i>	ND	ND	70	80	-	-	0.4 mL
<i>Candida albicans</i>	70	80	-	-	60	70	0.4 mL

ND= Not detected, VG= Visible growth

3.2. In vitro biological assessment.

The plant kingdom symbolizes a vast reservoir of medicinally active components with different chemical structures and preventive disease. Volatile fruit oil from *X. aethiopica* was assessed for antimalarial potency against chloroquine-sensitive (CQs) 3D7 *P. falciparum*, against *T.b. brucei*, a trypanosome accountable for nagana and cytotoxicity through the human cervix adenocarcinoma (HeLa) cell line. For comparative purposes, the positive control adopted for these assays are chloroquine for *P. falciparum*, pentamidine for *T. b. brucei*, and emetine for HeLa cell, and the screening assays were Plasmodium Parasite Dehydrogenase (*pLDH*) for malaria, *T. b. brucei*, and HeLa cells.

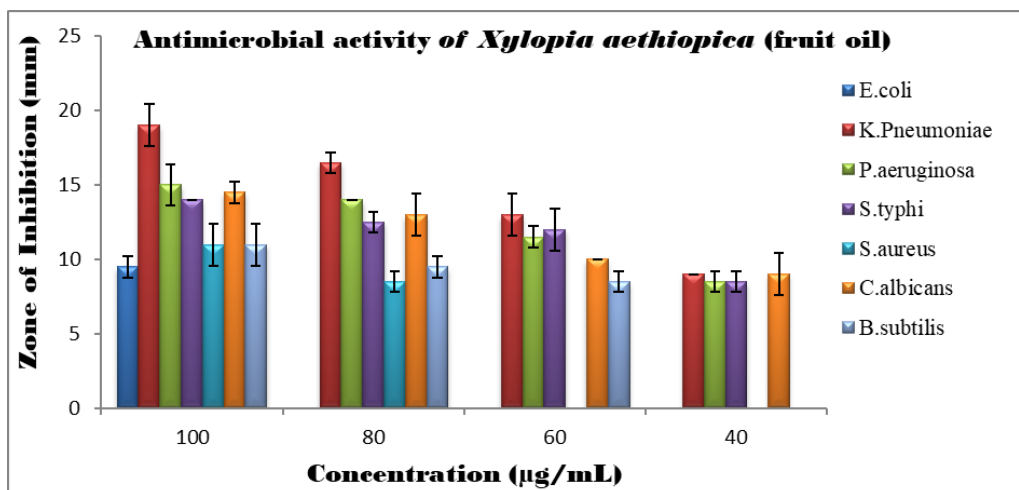


Figure 1. Antimicrobial activity of *Xylopia aethiopica*.

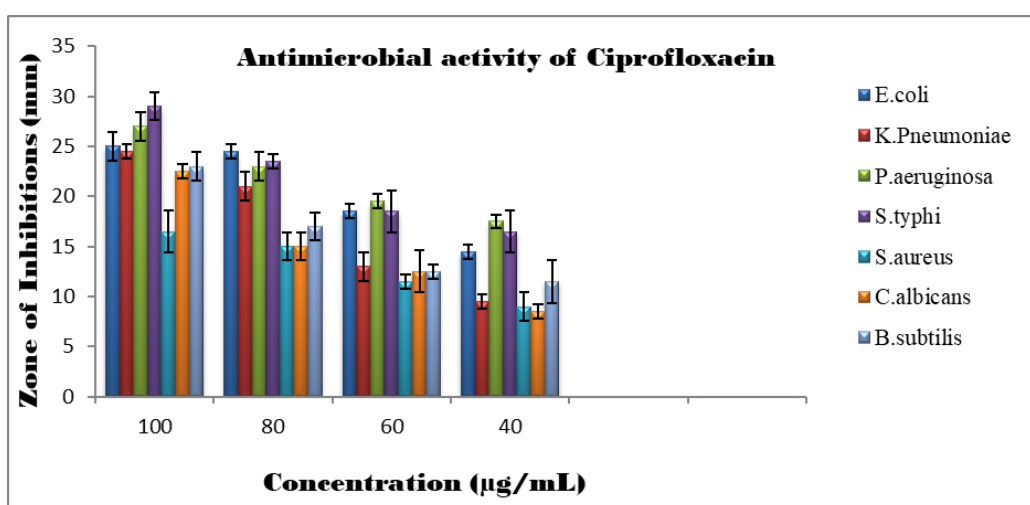


Figure 2. Antimicrobial activity of ciprofloxacin.

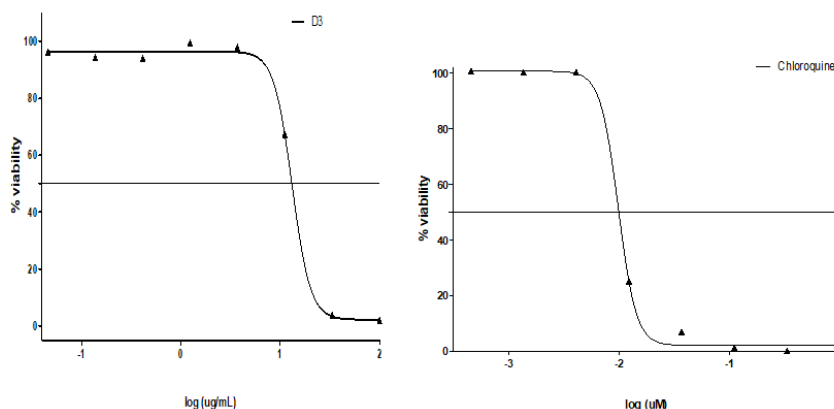


Figure 3. Dose-response assay for *X. aethiopica* and chloroquine.

The volatile oil of *X. aethiopica* only reduced the viability of trypanosomes (*T. b. brucei*) to $83.38 \pm 2.65\%$, samples or extracts that are not capable of bringing a considerable reduction to about 20% of the trypanosome parasites are considered not active, therefore the fruit oil of *X. aethiopica* was inactive against trypanosome parasites of *T. b. brucei*. Usually, herbal medicine is believed to be safer; for this reason, little research is carried out to test for the safety or toxicity of plant extracts; conversely, the toxicity test was conducted on the volatile fruit oil. The oil showed no cytotoxicity effects because the percentage viability was

91.99 ± 8.31% as determined by the viability of HeLa cells at a concentration of 50 µg/mL, the oil was not capable of reducing the viability of HeLa cells to below 50%; this might be an indication of their safety as targeted drugs for mammalian organisms while the % viability of the volatile fruit oil against 3D7 strain of *P. falciparum* was 6.67 ± 7.74%, this caused a considerable reduction in *pLDH* activity to less than 20 %, the result necessitated the volatile fruit oil to be put forward for *pLDH* IC50 screening by plotting the % viability against the volatile oil concentration using a threefold dilution, the IC50 for the oil was generated from the resulting dose-response curve by non-linear regression. The IC50 obtained for the volatile fruit oil was 14 µg/mL showing good activity against the parasite *P. falciparum*. Bero *et al.* [42] posited that extracts with good activity always demonstrate an IC50 value of less than 20 µg/mL, while extract which displays IC50 in the range of 20-60 µg/mL have moderate activity but those with IC50 above 100 µg/mL are inactive.

The antimalarial activity of this oil may be traced to the various constituents such as monoterpenes hydrocarbons (58.0%), oxygenated monoterpenes (30.13%), sesquiterpene hydrocarbons (8.46%), and fatty acids (0.74%) found in the crude volatile of the oil since it has been documented by some researchers that terpenes compound are accountable for the antimalarial potencies of many therapeutic plants [43], Schmidt *et al.* [44] also posited that important class of antiplasmodial natural products are the lower molecular weight constituents of volatile oils (VOs), such as monoterpenes, sesquiterpenes and phenylpropanoids [45], and this can be seen in the volatile fruit oil constituents of *X. aethiopica* Table 1. Consequently, VOs with a high amount of monoterpene constituents usually display antiplasmodial activity [44]. Notably, mechanistic studies have shown that monoterpenes that have low molecular weight like limonene and linalool, and sesquiterpenes, such as farnesol and nerolidol, stalls the biosynthesis of bioactive metabolites like dolichols and the terpenoids side chains of ubiquinones in the trophozoite and/or schizont stages of *Plasmodium falciparum* [46].

The antimicrobial screening of the volatile fruit oil of this plant as shown in Figures 1 & 2 demonstrates that *X. aethiopica* fruit oil exhibits antimicrobial properties against both Gram-negative, Gram-positive bacteria and the fungus used for this study; in addition to this, the broad spectrum of activities of the microorganisms used was also established, this, therefore, pave the way for a subsequent antimicrobial test such as MIC and MBC/MFC. The volatile fruit oil extract has a significant zone of inhibitions against all the seven test organisms. *K. pneumoniae* had the highest inhibition zone of 19.0 ± 1.4 mm followed by *P. aeruginosa* at 15.0 ± 1.4 mm, *C. albicans* at 14.5 ± 0.7 mm and *S. typhi* at 14.0 ± 0.0 mm, the least is *E. coli* at 9.5 ± 0.7 mm. The poor activity recorded for *E. coli* in Figure 2 substantiates previous reports documented by some researchers on the low sensitivity of volatile fruit oil of *X. aethiopica* on Gram-negative bacterium *E. coli* [25, 28]. The activities of the tested microorganisms compare favorably well with that of the standard antibiotic drug ciprofloxacin. The minimum inhibitory concentration was carried out to ascertain the minimum concentration of the oil that inhibited the growth of the microbes used in this study. The MIC helps determine the level of the resistance of a particular bacteria strain. Same MIC values of (80 µg/mL) were recorded for *P. aeruginosa*, *S. typhi*, and *B. subtilis* while the MIC values of *K. pneumoniae* and *C. albicans* were the same (70 µg/mL), showing that they were more active than *P. aeruginosa*, *S. typhi*, and *B. subtilis* Table 2. The MBC/MFC was carried out to ascertain whether the volatile oil actually killed the test pathogens or that their growths were only inhibited by the essential oil. The results indicated that *P. aeruginosa*, and *C. albicans* were killed at a concentration of 80 µg/mL while *K. pneumoniae*, *S. typhi*, and *B. subtilis* were

killed at a concentration of 90 µg/mL (Table 2). In traditional settings, several ailments are treated with the aerial parts of *X. aethiopica*, such as dysentery and biliousness, cough, bronchitis, boils, and sores [12-13]. Documentation from previous researches has shown that *X. aethiopica* exhibits various biological activities like antimicrobial, anti-asthmatic, anti-inflammatory, anti-tumor, hypotensive, and coronary effects ascribed to the different secondary metabolites in the plant [47].

The result of MBC/MFC proves that the volatile fruit oil from this plant may be used in therapeutic preparations, especially in the diseases caused by some of these microorganisms as practiced by traditional healers in folkloric medicines. This seems to justify the numerous folkloric medicinal uses of the plant [10, 12-16, 19].

4. Conclusions

This present investigation on the volatile fruit oil of this plant showed that the fruit is a potentially good source of traditional medicine. Because it is a medicinal plant, it has a commercial interest in both research institutes and pharmaceuticals company for the manufacture of new drugs in the treating of various diseases. The result of MBC/MFC proves that the essential oil of this plant can be used in therapeutic preparations, especially in the diseases caused by some of these microorganisms used. In addition to this, the volatile oil from this plant could be used as an excellent substitute to synthetic antiplasmodial and antimicrobial therapeutic drugs to battle malaria and infectious ailment from various bacterial strains.

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Conflicts of Interest

The authors declare no conflict of interest.

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